

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claim 45 (previously presented): An in vitro method for determining the functional activity of one or more components of the Protein C anticoagulant pathway of the blood coagulation system, comprising:

- (a) providing a blood sample to be analyzed;
- (b) activating the coagulation cascade by adding a procoagulant reagent to the blood sample to be analyzed;
- (c) triggering coagulation by adding calcium ions to the blood sample;
- (d) adding metal ions selected from the group consisting of Mg^{+2} , Mn^{+2} , Zn^{+2} , Ni^{+2} , Sr^{+2} , Cu^{+2} , or Cu^{+} , ions at a concentration that increases the anticoagulant activity of one or more components of the Protein C anticoagulant pathway;
- (e) incubating a reaction mixture comprising the components recited in steps (a)-(d);
- (f) observing clotting time; and
- (g) comparing the clotting time for the blood sample to be analyzed with the clotting time for a normal blood sample as determined by the method recited in steps (a)-(f), thereby allowing for determination of an activity of one or more components of the Protein C anticoagulant pathway.

Claim 46 (previously presented): The method according to claim 45, wherein the metal ion comprises Mg^{2+} .

Claim 47 (currently amended): The method according to claim 46, wherein the metal ion comprises Mg^{2+} and the amount of the Mg^{2+} ions added in step (d) is about 20 μmol to about 10 mmol per liter of reaction mixture.

Claim 48 (currently amended): The method according to claim 46, wherein the metal ion comprises Mg^{2+} and the amount of the Mg^{2+} ions added in step (d) is about 100 μmol to 2 about mmol per liter of reaction mixture.

Claim 49 (currently amended): The method according to claim 46, wherein the metal ion comprises Mg^{2+} and the amount of the Mg^{2+} ions added in step (d) is about 200 μmol to about 1 mmol per liter of reaction mixture.

Claim 50 (currently amended): The method according to claim 45, wherein the amount of Mn^{+2} , Zn^{+2} , Ni^{+2} , Sr^{+2} , Cu^{+2} , or Cu^{+} ions added in step (d) is about 1 μmol to about 2 mmol per liter of reaction mixture.

Claim 51 (currently amended): The method according to claim 45, wherein the amount of Mn^{+2} , Zn^{+2} , Ni^{+2} , Sr^{+2} , Cu^{+2} , or Cu^{+} ions added in step (d) is about 5 μmol to about 400 μmol per liter of reaction mixture.

Claim 52 (currently amended): The method according to claim 45, wherein the amount of Mn^{+2} , Zn^{+2} , Ni^{+2} , Sr^{+2} , Cu^{+2} , or Cu^{+} ions added in step (d) is about 10 μmol to about 80 μmol per liter of reaction mixture.

Claim 53 (previously presented): The method according to claim 45, wherein the blood sample is selected from the group consisting of whole blood, blood plasma, and blood serum.

Claim 54 (previously presented): The method according to claim 45, wherein the activating, triggering, and adding steps occur separately.

Claim 55 (previously presented): The method according to claim 45, wherein the activating, triggering, and adding steps occur simultaneously.

Claim 56 (currently amended): The method according to claim 45, wherein the amount of calcium ions added in step (c) is about 0.5 mmol to about 20 mmol per liter of reaction mixture.

Claim 57 (currently amended): The method according to claims 45, wherein the amount of calcium ions added in step (c) is about 1 mmol to about 10 mmol per liter of reaction mixture.

Claim 58 (currently amended): The method according to claims 45, wherein the amount of calcium ions added in step (c) is about 200 μ mol to about 1 mmol per liter of reaction mixture.

Claim 59 (currently amended): The method according to claim 45, wherein the activating the coagulation cascade step occurs via the intrinsic pathway and the ~~precoagulation~~ procoagulant reagent comprises at least one phospholipid and at least one contact activator.

Claim 60 (currently amended): The method according to claim 45, wherein the activating the coagulation cascade step occurs via the intrinsic pathway and the ~~precoagulation~~ procoagulant reagent comprises at least one phospholipid and at least one intrinsic pathway factor selected from the group consisting of Factor IXa, Factor XIIa, and Factor XIa.

Claim 61 (currently amended): The method according to claim 45, wherein the activating the coagulation cascade step occurs via the intrinsic pathway and the ~~precoagulation~~ procoagulant reagent comprises at least one phospholipid, at least one contact activator and at least one intrinsic pathway factor selected from the group consisting of Factor IXa, Factor XIIa, and Factor XIa.

Claim 62 (previously presented): The method according to claim 61, wherein the at least one contact activator is selected from the group consisting of ellagic acid, collagen, collagen-related substances, and silica.

Claim 63 (previously presented): The method according to claim 62, wherein the at least one contact activator is a silica selected from the group consisting of micronized silica, colloidal silica, and kaolin.

Claim 64 (currently amended): The method according to claim 45, wherein the activating the coagulation cascade step occurs via the extrinsic pathway and the ~~precoagulation~~ procoagulant reagent comprises a material selected from the group consisting of native human tissue factor, recombinant human tissue factor, non-human native tissue factor, non-human recombinant tissue factor, native human Factor VII/VIIa, recombinant human Factor VII/VIIa, native non-human Factor VII/VIIa, and recombinant non-human Factor VII/VIIa.

Claim 65 (previously presented): The method as in any one of claims 59-61, wherein the at least one phospholipid is selected from the group consisting of synthetic phospholipids, purified phospholipids, and crude extracts of phospholipids derived from biological sources.

Claim 66 (previously presented): The method according to claim 65, wherein the at least one phospholipid is selected from the group consisting of phosphatidylcholine, phosphatidylserine, and sphingomyelin.

Claim 67 (previously presented): The method according to claim 45, wherein the activating the coagulation cascade step occurs via the common pathway and the procoagulation reagent comprises a material selected from the group consisting of exogenous Factor Xa, exogenous Factor X and an exogenous activator for Factor X, and an exogenous activator for endogenous Factor X.

Claim 68 (previously presented): The method according to claim 67, wherein the exogenous activator for Factor X comprises snake venom enzyme.

Claim 69 (previously presented): The method according to claim 68, wherein the exogenous activator for Factor X comprises Russell's Viper snake venom enzyme.

Claim 70 (previously presented): The method according to claim 45, further comprising the step of adding at least one component selected from the group consisting of Protein C, activated Protein C, Protein S, Factor V, Factor Va, a plasma deficient of

the Protein C anticoagulant pathway component to be analyzed, and a plasma deficient of all components of the Protein C anticoagulant pathway.

Claim 71 (previously presented): The method according to claim 45, wherein a fibrin polymerization inhibitor is added to the blood sample to be analyzed.

Claim 72 (previously presented): The method according to claim 71, wherein the fibrin polymerization inhibitor comprises Gly-Pro-Arg-Pro.

Claim 73 (currently amended): The method according to claim 45, wherein the ~~procoagulation~~ procoagulant reagent comprises a material selected from the group consisting of Factor VIII, Factor VIIIa, Factor IX, Factor X, and prothrombin.

Claim 74 (previously presented): The method according to claim 45, the method further comprising the step of providing activated Protein C by adding exogenous activated Protein C to the blood sample to be analyzed.

Claim 75 (previously presented): The method according to claim 45, the method further comprising the step of providing activated Protein C by adding an activator of Protein C to the blood sample to be analyzed.

Claim 76 (previously presented): The method according to claim 45, the method further comprising the step of providing activated Protein C by adding exogenous Protein C together with an activator of Protein C to the blood sample to be analyzed.

Claim 77 (previously presented): The method as in any one of claims 74-76, wherein the adding metal ions step occurs simultaneously with the providing activated Protein C step.

Claim 78 (previously presented): The method as in any one of claims 74-76, wherein the providing activated Protein C step occurs simultaneously with the activating the coagulation cascade step.

Claim 79 (previously presented): The method as in any one of claims 74-76, wherein the providing activated Protein C step precedes the activating the coagulation cascade step.

Claim 80 (previously presented): The method as in any one of claims 74-76, wherein the Protein C activator comprises at least one substance selected from the group consisting of Protein C activating snake venom enzyme and thrombin.

Claim 81 (previously presented): The method as in any of claims 74-76, wherein the Protein C activator comprises thrombomodulin.

Claim 82 (previously presented): The method as in any one of claims 74-76, wherein the Protein C activator comprises recombinant Protein C activator.

Claim 83 (previously presented): The method according to claim 80, wherein the Protein C activating snake venom enzyme is obtained from the Agkistrodon family.

Claim 84 (previously presented): The method according to claim 83, wherein the amount of purified Protein C activator added is about 2×10^{-3} U to 0.3 U per milliliter of reaction mixture.

Claim 85 (previously presented): The method according to claim 83, wherein the snake venom enzyme is obtained from Agkistrodon contortrix contortrix.

Claim 86 (previously presented): The method according to claim 83, wherein the snake venom enzyme comprises crude snake venom enzyme.

Claim 87 (previously presented): The method according to claim 83, wherein the snake venom enzyme comprises purified snake venom enzyme.

Claim 88 (currently amended): The method according to claim 87, wherein the amount of purified snake venom enzyme is about 1×10^{-3} U to about 1 U per milliliter of reaction mixture.